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His principal research interests include studies on the nutritional value of proteins and fats, the chemistry of food flavors and the role of vitamin B_{12} in nucleic acid metabolism. An able and prolific writer, as well as a researcher, he is the author of approximately 150 scientific publications in the field of biochemistry and nutrition, and meat research.

All of Professor Schweigert's undergraduate and graduate work was taken at the University of Wisconsin. He received the B.S. degree (1942) and the M.S. degree (1944) in Biochemistry; in 1946 he received the Ph.D. degree in Biochemistry and Physiology under the direction of Dr. C. A. Elvehjem. During his undergraduate days, he was awarded scholarships in 1938 and 1940 and the Wisconsin Alumni Research Foundation Research Apprenticeship for 1941-1942. He was named the outstanding senior in the College of Agriculture in 1942. While doing his graduate work, he had a research-teaching assistantship at the University of Wisconsin from 1942-1946 and the National Live Stock and Meat Board Fellowship from 1943-1945.

Honorary Societies in which Dr. Schweigert has been awarded membership include Phi Eta Sigma, Phi Kappa Phi, Alpha Zeta, Phi Lambda Upsilon, Gamma Alpha and Sigma Xi. He also is a member of these professional societies: American Society of Biological Chemists, American Institute of Nutrition, Society of Experimental Biology and Medicine, American Chemical Society, Society of American Bacteriologists, American Society of Animal Production, American Association for the Advancement of Science, Institute of Food Technology and the American Dairy Science Association.

Two years (1946-1948) as Associate Professor of Biochemistry and Nutrition at Texas Agricultural and Mechanical College preceded his appointment as Chief of the Division of Biochemistry and Nutrition, the American Meat Institute Foundation. In 1953 he was appointed Assistant Director of Research and Education and then in 1956 he was appointed Director of Research and Education. Concurrent with his work at the American Meat Institute Foundation he was an Assistant Professor (1948-1951) and then an Associate Professor (1951-1960) in the Department of Biochemistry, The University of Chicago. In 1960 he was appointed to his present position at the College of Agriculture, Michigan State University.

Never too busy to help further his professional field, he has accepted national appointments to the subcommittee on nutritional requirements of laboratory animals and the subcommittee on Irradiation of Foods of the National Research Council. He also is chairman of the Committee on Irradiation Preservation of Foods of the American Institute of Biological Sciences and the subcommittee on Animal Products, the National Research Council.

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The Role of Vitamin B_{12} in Nucleic Acid Synthesis

B. S. Schweigert, Ph.D.

INTRODUCTION

Two of the B vitamins, folic acid and vitamin B_{12}, have a unique role in the metabolism of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). While detailed mechanisms are not yet worked out, RNA has a key role in protein synthesis and DNA is the prime constituent of genes and chromosomes associated with cell division.

Vitamin B_{12} functions in man in the prevention of pernicious anemia. For many years prior to the discovery of vitamin B_{12}, liver preparations injected intramuscularly were used for the treatment of this disease. Then came the important observations that the growth-factor potency of liver extracts for the microorganism, *Lactobacillus lactis Dorner,* correlated well with the curative-potency when used in the treatment of pernicious anemia. Using this knowledge, rapid progress was made in the late 1940's in the isolation and identification of vitamin B_{12}. In other laboratories, the red color of vitamin B_{12}, coupled with clinical tests, also helped in the isolation of vitamin B_{12}.

DEFICIENCY SYMPTOMS

The fundamental defect in pernicious anemia appears to be faulty absorption of vitamin B_{12} from the gastro intestinal tract of man. It is correctable by oral administration of an "intrinsic factor" preparation from hog stomach, for example, plus oral vitamin B_{12}, or by the injection of vitamin B_{12} which circumvents the absorption problem. It is of interest that folic acid therapy will often correct the anemia symptoms of pernicious anemia but effective vitamin B_{12} treatment is needed to correct both the anemia and the neurological symptoms of this disease.

For sometime it was difficult to demonstrate a clear cut need for vitamin B_{12}. Weanling rats fed the traditional type of diets successful in detecting the need for other B vitamins did not work for vitamin B_{12}. However, eventually it was found that a vitamin B_{12} deficiency could be produced by either feeding a vitamin B_{12} deficient ration to the mothers during pregnancy and lactation or by incorporating a thyroid stimulant, such as iodinated casein, into the ration of the weanling rat. A deficiency can be produced in the chick without special methods with the use of purified feed ingredients and plant protein preparations.

SOURCES

The occurrence of vitamin B_{12} is unique among vitamins in that it is absent in higher plants or occurs at a level below which it can be detected with certainty. Vitamin B_{12} is found almost exclusively in foods of animal origin and much of this occurs in the form of a protein complex. This fact led to the early description

of the nutrient as the "animal protein factor" since liver and milk, for example, are rich sources of this vitamin.[18] The synthesis of vitamin B_{12} in nature, therefore, is by microorganisms. It is produced commercially by fermentation. The wide occurrence of vitamin B_{12} in foods of animal origin presumably supplies ample quantities of this vitamin for the human population. A need for a supplement of this vitamin has been demonstrated only for individuals with certain macrocytic anemias.

CHEMICAL NATURE

Vitamin B_{12} also has a unique chemical composition in that it contains the element cobalt coordinated with 4-nitrogen-containing pyrol groups, a benzimidazole nucleotide moiety, and various substituent groups attached to the cobalt. It also may exist in several forms. The parent compound is termed cobalamin, and the analogs are called cyanocobalamin (vitamin B_{12}), hydroxyxobalamin (vitamin B_{12a} and vitamin B_{12b}) sulfitacobalamin etc., depending on the group attached to the cobalt. Several "pseudovitamin B_{12} compounds" are known in which a purine base, such as adenine, has replaced the benzimidazole group (pseudovitamin B_{12f}). These compounds have varying vitamin B_{12} activity for microorganisms but are essentially inactive for man and animals. Thus, they are ineffective in the treatment of patients with pernicious anemia. An interesting new derivative has recently been described as a coenzyme form of vitamin B_{12}. It functions in the interconversion of glutamate and beta-methyl-aspartate by bacteria.[23] Chemical studies indicate that this compound differs from pseudovitamin B_{12} in that it contains two adenines with the second adenine-containing constituent attached to the cobalt in place of cyanide.

METABOLIC ROLES

The early studies provided valuable background nutritional information which served as guides in planning later studies to determine the role of vitamin B_{12} in metabolism. In the early 1950's, we had several clues indicating the probable role of vitamin B_{12} in metabolism, including possible relationships to nucleic acid metabolism. The main observations at that time included:

1. The vitamin B_{12} requirement for the most commonly used assay organism, *Lactobacillus leichmannii,* also could be met by a deoxynucleoside (component of DNA), such as thymidine.
2. The vitamin requirement was reduced by use of sulfhydryl compounds (cysteine) and the level of compounds of this type in the blood and liver of animals fed vitamin B_{12} deficient diets was altered. This suggested the "sparing" action of sulfhydryl compounds for vitamin B_{12}.
3. High levels of the amino acid, glycine, were toxic to vitamin B_{12} deficient chicks.
4. The action of vitamin B_{12} appeared to be closely related to folic acid in that animals deficient in both these vitamins had a reduced

ability to methylate homocysteine or betaine to form methionine and choline, respectively.

5. The vitamin functioned in correcting certain macrocytic anemias in man, including pernicious anemia.

The apparent metabolic roles of the vitamin, as suggested by these nutritional observations, may be summarized as (1) nucleic acid metabolism, (2) amino acid metabolism, (3) oxidation-reduction reactions of dithio groups, and (4) one carbon metabolism, such as in methylation reactions. Obviously, the specific biochemical role may be common to all of these reactions. This can be determined only after reactions in specific enzyme systems have been characterized.

NATURE OF NUCLEIC ACIDS

Nucleic acids are complex substances with molecular weights in the millions in many cases and are made up on nucleotide units. Ribonucleotides (of RNA) are composed of a purine or pyrimidine base, the 5-carbon sugar, ribose, and a phosphate group esterified to ribose. For example, the ribonucleotide, adenylic acid, may be written adenine-ribose-phosphate. The nucleotides are linked together in RNA through phosphate ester groups. The four major nucleotide units in RNA are adenylic acid, guanylic acid, cytidylic acid and uridylic acid which differ only in the nature of the purine or pyrimidine base present. Nucleosides are composed of the sugar and purine or pyrimidine base without the phosphate group.

Deoxynucleotides (of DNA) differ from the corresponding ribonucleotides in having the sugar, deoxyribose, present instead of ribose. Deoxyribose differs from ribose in chemical structure by a hydrogen replacing a hydroxyl group on carbon atom number two. Another major difference is that the pyrimidine base, thymine, occurs in DNA instead of uracil. An example of a deoxynucleotide is deoxyadenylic acid (adenine-deoxyribose-phosphate).

The interesting observation that the chemically dissimilar compound, thymidine (thymine deoxyriboside), also would fulfill the vitamin B_{12} requirement for *Lactobacillus leichmannii,*[8] but not for *Englena gracilis,* stimulated research on the possible role of this vitamin in nucleic acid metabolism. From a comparative viewpoint, another microorganism, *Lactobacillus acidophilus* R-26, has a requirement for a deoxynucleoside, such as thymidine, that is not fulfilled by vitamin B_{12}.[6]

These facts, suggested from nutritional experiments, prompted us and other laboratories to investigate the possible role of vitamin B_{12} in nucleic acid synthesis by the rat and by microorganisms.

ROLE IN NUCLEIC ACID SYNTHESIS IN RATS

In studies with the rat, the first experiments were carried out to determine the nucleic acid composition of livers from vitamin B_{12} deficient and supple-

mented rats. As shown in Tables I-III, a significant reduction in the DNA and RNA contents occurred in the livers of the deficient rats. There were no differences observed, however, in the amounts of these constituents in the nuclei of individual liver cells.[16] The ability of the deficient rats to regenerate liver also

TABLE I.

Effect of Vitamin B_{12} Deficiency on the Nucleic Acid Content of the Liver

Dietary Regimen*	$-B_{12}$	$+B_{12}$
No. of Rats	7	7
Gm. gain/week	20.2	35.0
DNA (mg/gm)	2.39	2.99
RNA (mg/gm)	5.13	6.91

*For a four-week period. The $+B_{12}$ group received 24 ug of vitamin B_{12} per kg. of ration.

TABLE II.

Effect of Vitamin B_{12} on the DNA Content of the Liver

Dietary Regimen	Body Weight gm	No. nuclei/gm ($\times 10^6$)	DNA	
			mg/gm	mg/cell ($\times 10^{-8}$)
B_{12} deficient	170	138	2.04	1.49
B_{12} supplemented	240	170	2.58	1.54
$+B_{12}$ (food restricted)	172	176	2.67	1.52

TABLE III.

Liver Regeneration of Rats Fed Iodinated Casein Diets After Partial Hepatectomy

	Basal				Basil + 50ug. Vit. B_{12}/kg.			
	Before	O Time	4 days	Percent-Regeneration	Before	O Time	4 days	Percent-Regeneration
Wt. gm.	13.9	6.4	9.4	40	12.2	4.5	10.2	74
RNA, mg.	70	32	53	55	67	25	60	83
DNA, mg.	27.5	12.7	18.9	42	30.8	11.5	25.9	75

was impaired after partial hepatectomy.[19] Thus, it could be concluded that the number of cells in the livers was reduced per unit of weight, of dry weight or of nitrogen in the vitamin B_{12} deficient animals. These studies provided promising leads for further experiments with isotopic tracers in attempts to learn more about nucleic acid metabolism in the rat.

Studies by Rose[17] have shown that totally labeled cytidine (C^{14}) is converted to pyrimidine deoxyribosides of DNA without cleavage of the sugar from the base prior to incorporation by the rat (Table IV). Intact incorporation of

TABLE IV.

Incorporation of Totally Labeled Cytidine into Nucleosides of Rat DNA*

Test Compound** (cpm/ug carbon)	Specific Activity of Deoxyribosides in cpm/ug of carbon				
	C of Cdr	dr of Cdr	T of Tdr	dr of Tdr	dr of Gdr + Adr
Cytidine (40)	.15	.14	.12	.07	.007

*C=Cytosine, dr=deoxyribose, T=thymine, G=Guanine, A=Adenine

**80 uM/100 gms body weight injected subcutaneously.

TABLE V.

Incorporation of Totally Labeled Cytidine into Nucleosides of Rat RNA**

Test Compound* (cpm/ugm) carbon)	Specific Activity of Ribosides in cpm/ugm of carbon					
	C of Cr	r of Cr	U of Ur	r of Ur	r of Gr	r of Ar
Cytidine (40)	.88	.95	.47	.18	.01	.02

*80 uM/100 gms body weight injected subcutaneously.

**See abbreviations, Table IV. r=ribose, U=Uracil

cytidine into RNA is shown in Table V. These experiments, it is recognized, did not show if the conversion of the ribosides to deoxyribosides occurs at the nucleoside level or at some more complex state. Recent experiments by Reichard[14, 15] show that this transformation occurs at the nucleotide level with chick embryos and *Escherichia coli.*

Subsequent experiments were conducted by injecting radioactive cytidine into vitamin B_{12} deficient and supplemented rats. The results (Table VI) indicate

TABLE VI.

Radioactivity of Ribonucleotides Isolated from Rat Liver Expressed as CPM/uM

Regimen	base	Sugar/base*
$+12_{12}$		
Cytidylic acid	142	1.87
	157	1.90
Uridylic acid	76	0.62
	76	0.49
$-B_{12}$		
Cytidylic acid	127**	2.50
	228	2.60
Uridylic acid	87**	0.76
	160	0.48

*Ratio for injected cytidine=2.6

**Mildly deficient

less dilution of the radioactivity occurred in the pyrimidine nucleotides of RNA in the most deficient animal as compared with the controls.[1] Unfortunately, in these studies the incorporation of vitamin B_{12} into DNA was at too low a level to ascertain if the vitamin influences the conversion of ribosides to deoxyribosides. An interesting aspect of this work, however, was the radioactivity of the uridylic acid obtained from the liver RNA. The sugar-base ratio was not the same as for the injected or isolated cytidine. In this case, some of the injected cytidine was cleaved prior to incorporation into the uridylic acid of RNA. It appears that further elucidation of the role of vitamin B_{12} in nucleic acid metabolism of the rat might be accomplished by following nucleic acid biosynthesis in partially hepatectomized animals.

ROLE IN NUCLEIC ACID SYNTHESIS IN MICROORGANISMS

Studies have shown that vitamin B_{12} functions in the biosynthesis of the deoxyribose moiety of the DNA in *L. leichmannii*.[4] This finding indicates vitamin B_{12} is involved in nucleic acid metabolism. This is shown in Table VII. No dilution of the radioactivity in the deoxyribose of thymidine and the other nucleosides occurred when thymidine alone was provided to the microorganisms. However, when vitamin B_{12} was supplied along with the thymidine in the medium, decreased radioactivity occurred in the deoxyribose moiety in all cases. This indicates there is a greater nucleoside synthesis when vitamin B_{12} is supplied

TABLE VII.

Effect of Vitamin B_{12} on Deoxyriboside Synthesis by *L. Leichmannii* (Data expressed as CPM/uM)

Treatment	Compound Isolated*	Base	Sugar
Starting Material	Tdr	3030	3030
Tdr (1 mg.)	Tdr	280	3380
	Cdr	20	3300
	Adr	20	3900
	Gdr	50	2750
Tdr + 2 ug B_{12}	Tdr	355	65
	(All others too low to measure)		

*For abbreviations see Table IV.

in the medium. Cultures of this organism show a filamentous morphology when grown in the presence of subminimal levels of either vitamin B_{12} or thymidine.[2] These findings show an interesting correlation with the larger cells observed in the livers of vitamin B_{12} deficient rats.[13] These findings have been extended in recent studies done in other laboratories. Manson[9] has shown from radioactivity experiments that in the presence of vitamin B_{12}, the ribose part of a riboside acts as a precursor of the deoxyribose part of a deoxyriboside; this does not occur in the absence of vitamin B_{12}. Studies by Spell and Dinning[21, 22] have also indicated that vitamin B_{12} functions in the conversion of ribose to deoxyribose, as well as in the incorporation of formate into the methyl group of thymine in *L. leichmannii.*

These studies provide excellent evidence that one of the functions of vitamin B_{12} is the biosynthesis of the deoxyribose moiety of DNA. The net effect of converting a riboside to a deoxyriboside is the substitution of a hydrogen atom for a hydroxyl group at carbon number two in the sugar molecule. Thus, it is tempting to speculate that vitamin B_{12} may function in the conversion of a riboside to a deoxyriboside at the nucleoside or nucleotide level through some "reduction" mechanism. Clarification of this mechanism awaits the development of specific enzyme systems requiring a vitamin B_{12} derivative as a co-factor. Data on levels of RNA in livers of rats fed a deficient amount of vitamin B_{12} suggest that the vitamin also may function in the synthesis of this material. The effect could be through purine synthesis, as yet not well defined, or through some quite indirect mechanism.

Subsequent studies have shown that *L. leichmannii* require a purine base (guanine) in addition to a deoxyriboside when vitamin B_{12} is absent from the

TABLE VIII.

Acid Production by *Lactobacillus Leichmannii* with the Addition of Purine and Pyrimidine Bases in the Presence of Vitamin B_{12} or Thymidine

Bases added* (0.1 mg each/tube)	*L. leichmannii*, Strain 313	
	5 mug vitamin B_{12} added	5 ug thymidine added
AGU	10.9	9.2
GU	10.7	8.8
AG	9.0	7.8
G	8.7	7.9
AU	7.7	0.2
A	7.4	0.3
U	8.7	0.7
None	8.1	1.2

*For abbreviations see Tables IV and V.

medium.[3] As noted in Table VIII, adenine is inactive in this respect. When purine and pyrimidine bases are added to the medium containing no vitamin B_{12} all the deoxyribosides are equally active as suppliers of combined deoxyribose (Table IX).[11] Guanine, hypoxanthine and xanthine are active in providing the requirement for a purine base. As would be expected from these results, guanine deoxyriboside can meet the requirement for a purine base and deoxyriboside in the absence of vitamin B_{12}. Cytidine deoxyriboside, thymidine, or adenine deoxyriboside are inactive unless an active purine, such as guanine, is also present.

METABOLIC ROLES OF VITAMIN B_{12} DERIVATIVES

Determining the role of vitamin B_{12} derivatives in the metabolism of other substances may also provide further clues in nucleic acid metabolism. Studies of this type are being conducted and progress is being made.

The "coenzyme" form of vitamin B_{12}, already referred to, is involved in the interconversion of glutamate and beta-methyl-aspartate. Other studies show that this coenzyme or a related compound is effective in increasing the incorporation of amino acids (phenylalanine) in proteins,[10] and is involved in the metabolism of methyl malonyl coenzyme A (a coenzyme containing pantothenic acid that functions in fatty acid metabolism) to succinyl coenzyme A,[5, 20] and in the metabolism of pyruvate.[12] As yet, no apparent relationship of these reactions to nucleic acid metabolism is evident, although beta-methyl-asparate reduces the requirements for thymine.[7]

TABLE IX.

Activity of Deoxyribosides in the Presence of a Mixture of Adenine, Guanine, Uracil and Xanthine and in the Absence of Vitamin B_{12}

Compound*	maximum acid production (ml)	acid k, uM**
C dr	18.6	0.66
T dr	16.6	0.64
A dr	17.3	0.76
G dr	16.5	0.72

*For abbreviations see Table IV.
**k=number of micromoles required for half-maximum acid production.

Other studies, briefly referred to earlier, show the importance of vitamin B_{12} and folic acid in the metabolism of one-carbon compounds, such as formate, beta carbon of serine, alpha carbon of glycine etc. in methylation reactions. They do not provide, however, a direct clue to nucleic acid metabolism. Formate, the beta carbon of serine, the alpha carbon of glycine, and CO_2 are known to be precursors of specific carbon atoms of purines; however, the specific role of vitamin B_{12} in purine synthesis is not known.

SUMMARY

Since the nutritional observation was made that the needs of *L. leichmannii* for vitamin B_{12} are satisfactorily met by a deoxyribonucleoside, much progress has been made in elucidating the role of this vitamin in nucleic acid metabolism. The work reviewed illustrates two fundamental principles: (1) The value of nutritional observations in suggesting fundamental biochemical mechanisms and (2) The value of using a variety of experimental animals and microorganisms as experimental test organisms.

While much remains to be done, research thus far clearly demonstrates the role of vitamin B_{12} in the synthesis of the deoxyriboside moiety of DNA. It suggests, too, that this vitamin may function in the direct conversion of the ribosides of RNA to deoxyribosides of DNA by reduction at the number two carbon of the sugar molecule. The significance of studies on the role of this vitamin in the interconversion of glutamate and beta-methyl-aspartate, in incorporation of amino acids into protein, in the metabolism of one carbon and sulfhydryl compounds as related to nucleic acid metabolism cannot as yet be ascertained. In view of the basic significance of this vitamin in nutritional health and of nucleic acids to cell division phenomenon, we can anticipate continued attention to this important field.

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CORRECTION

In Dr. M. K. Horwitt's article "Vitamin E in Human Nutrition — An Interpretive Review" in the January - March, 1961 issue, page 10, line 6 of text copy, the word "tocopherols" should be replaced by "substitutes."

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